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TITLE: Pathogenesis and Prediction of Future Rheumatoid Arthritis

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14. ABSTRACT It is now well established that there is a preclinical period of rheumatoid arthritis (RA) development that is characterized by abnormalities of the immune system prior to the onset of the clinically apparent inflammatory joint disease that currently defines RA. The primary goal of this project is to investigate this preclinical period in order to understand two major factors: 1) how biomarker changes in preclinical RA can be used to accurately predict the future development of RA in currently asymptomatic individuals, and 2) to identify factors related to the pathogenesis of RA that can ultimately be targeted to prevent RA. This project has proposed to use a unique set of serum samples and clinical data available through the Department of Defense Serum Repository (DoDSR) to investigate the preclinical period of RA. During this project, we acquired the serum samples and data from the DoDSR, and completed biomarker testing. Because of delays in obtaining the final data from the DoDSR, we have requested a no-cost extension until 29-Sep-2017 during which final analyses will be performed.					
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Table of Contents

	<u>Page</u>
1. Introduction.....	3
2. Keywords.....	3
3. Accomplishments.....	3-6
4. Impact.....	6
5. Changes/Problems.....	6-7
6. Products.....	7
7. Participants & Other Collaborating Organizations.....	8-10
8. Special Reporting Requirements.....	11
9. Appendices.....	None

INTRODUCTION

This is an Annual Report for the project entitled “Pathogenesis and Prediction of Rheumatoid Arthritis”, PI Kevin D. Deane, period 30 Sep 2013 – 29 Sep 2016.

The date that this report is submitted is 3 Mar 2017. **Of note, this report is submitted at this time because the project has been granted a no-cost extension until 29 Sep 2017 in order to complete analyses that were delayed by delayed data release from the DoDSR.**

It is now well established that there is a preclinical period of rheumatoid arthritis (RA) development that is characterized by abnormalities of the immune system prior to the onset of the clinically apparent inflammatory joint disease that currently defines RA. The primary goal of this project is to investigate this preclinical period in order to understand two major factors: 1) how biomarker changes in preclinical RA can be used to accurately predict the future development of RA in currently asymptomatic individuals, and 2) to identify factors related to the pathogenesis of RA that can ultimately be targeted to prevent RA.

This project has proposed to use a unique set of serum samples and clinical data available through the Department of Defense Serum Repository (DoDSR) to investigate the preclinical period of RA.

As described below in more detail, over this 3-year project, we have acquired the serum samples and data from the DoDSR, and completed biomarker testing. There have been delays in obtaining the final set of clinical data from the DoDSR. As such, the final analyses for this project have been delayed. We now have the final data and are in the process of analyses, and will be submitting manuscripts/abstracts of our findings, with the estimated first submission to the American College of Rheumatology Annual Meeting in June 2017.

KEYWORDS

Pathogenesis of rheumatoid arthritis
Prediction of rheumatoid arthritis

ACCOMPLISHMENTS

What were the major goals of the project?

As stated in the Statement of Work (SOW) the major goals/tasks were as follows:

- 1) Clinical data and sample procurement from the DoDSR. (SOW Task 1)
- 2) Obtain regulatory IRB/HRPO approval. (SOW Task 2)
- 3) Research assistant hiring and training. (SOW Task 3)
- 4) RA-related autoantibody testing in 1600 serum samples from 200 RA cases and 200 healthy controls. The specific autoantibodies include testing for anti-cyclic citrullined peptide (anti-CCP)-2, anti-CCP3.1, rheumatoid factor isotypes, and an array for antibodies to citrullinated protein antigens

- (ACPAs). (SOW Task 4)
- 5) Testing antibodies to oral pathogens in 1600 serum samples from 200 RA cases and 200 healthy controls. (SOW Task 6).
 - 6) Testing serum samples for cotinine levels (SOW Task 7).
 - 7) Analyses related to Aims 2 and 3(SOW Tasks 8-9).
 - 8) Interim analyses and potential abstract preparation/submission (SOW Task 10).
 - 9) Final manuscript(s) preparation (SOW Task 11).
 - 10) Isotype testing to CCP (added 1-Oct-2014) (SOW Task 12).

What was accomplished under these goals?

Task 1. We obtained clinical data and serum samples from the DoDSR. These samples are now housed at the University of Colorado Denver in Dr. Deane's lab and the serum samples have used to perform the tests as listed below. Clinical data was obtained per medical chart review and includes the items listed in the original SOW as follows: subject age, gender, race, region of enlistment and military specialty, time of onset of RA and symptoms, classification criteria met for RA, medication use pre- and post-RA diagnosis. In addition, other medical illnesses and other environmental exposures such as smoking, periodontal disease were ascertained.

Task 2. Local and governmental IRB approvals, and HRPO approval, were obtained for this project.

Task 3. Research assistants were hired and trained for this project in the first year of this project and continued their work throughout the project. Please see the 'Participation' section below for details of these individuals.

Task 4. We completed serum sample testing for each of the following:

- Anti-CCP2 using ELISA kits (Axis-Shield) and established methodologies.
- Anti-CCP3.1 using ELISA kits (INOVA) and established methodologies.
- Rheumatoid factor isotypes (A, G and M) using ELISA kits (INOVA) and established methodologies.
- ACPA arrays

Task 6. We have completed testing for oral pathogens *Porphyromonas gingivalis*, *Prevotella intermedia* and *Fusibacterium nucleatum*. This was performed in the lab of Drs Ted Mikuls and Geoffrey Thiele using established protocols. Full analyses of these results are planned for year 3 of this project.

Task 7. We have completed serum cotinine testing.

Tasks 8-9 We have had delays in the final release from the DoDSR matching clinical data to serum sample results. However, the final data was released as of December 2016, and we are now in the process of performing analyses.

Tasks 10-11. We have had some delays in the final analyses because there was a delay in the final release from the DoDSR of the dataset linking the clinical data to the serum samples. However, the final data was released as of December 2016 and we are now in the process of preparing analyses, abstracts and manuscripts. We estimate the first abstract from these data will be submitted in June of 2017 for the American College of Rheumatology Annual Meeting.

Task 12. Isotype testing to CCP. This was an add-on task because we had additional funds. We have completed this testing.

What opportunities for training and professional development has the project provided? During Year 3 and beyond of the project this project has/will allow for early career rheumatologists including fellows and junior faculty to participate in scientific research. Specifically, Rheumatology Fellow Lindsay Kelmenson is now working on final analyses and she has been added to the list of Personnel (see below).

How were the results disseminated to communities of interest? Nothing to report although we expect the first abstracts/publications from this project to be submitted June 2017.

What do you plan to do during the next reporting period to accomplish the goals? We are in the process now of performing final analyses using the completed dataset.

IMPACT

What was the impact on the development of the principal discipline(s) of the project? Nothing to report at this phase of the project. However, we expect that the findings from preclinical RA will inform the field, and in particular be useful in future clinical trials for RA prevention.

What was the impact on other disciplines? Nothing to report at this phase of the project.

What was the impact on technology transfer? Nothing to report at this phase of the project.

What was the impact on society beyond science and technology? Nothing to report at this phase of the project.

CHANGES/PROBLEMS

Changes in approach and reasons for change There have been no substantive changes in the overall approach. However, we had initially allocated a certain amount of funds to obtain the serum samples from the DoDSR; however, we needed fewer funds than anticipated. We petitioned the DoD to use these remaining funds for additional testing of the serum samples; specifically, to test for isotypes immunoglobulin (Ig) A, IgG and IgM

to citrullinated proteins (CCP). We obtained approval to complete this testing, and we have completed this testing.

Actual or anticipated problems or delays and actions or plans to resolve them Due to funding cut-backs at the DoD level as well as military-based issues with personnel who were able to procure clinical data from the DoD, we have had delays in obtaining the clinical data from our subjects. However, we have surmounted these issues and the full clinical data was released December 2016, allowing for completion of analyses as planned.

Changes that had a significant impact on expenditures Please see 'Changes in Approach' above.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents Nothing to report.

Significant changes in use or care of human subjects Nothing to report.

Significant changes in use or care of vertebrate animals Nothing to report.

Significant changes in use of biohazards and/or select agents Nothing to report.

PRODUCTS

Publications, conference papers, and presentations All analyses and resultant publications are planned for December 2017-June 2017; as such at this time, there is nothing to report.

Journal publications Nothing to report.

Books or other non-periodical, one-time publications Nothing to report.

Other publications, conference papers, and presentations Nothing to report.

Website(s) or other Internet site(s) Nothing to report.

Technologies or techniques Nothing to report.

Inventions, patent applications, and/or licenses Nothing to report.

Other Products Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project? There are no changes related to the original investigators on the project as reported in the original proposal with the exception that research assistants have been named for the project. The investigators and research assistants are as follows (and listed in alphabetical order after the PI). All 'Person Months Worked' are a summary for the entire project.

Name	Kevin D. Deane, MD/PhD
Project Role	PI
Nearest Person Month Worked	6
Contribution	Oversee the entire project
Funding Support	This project

Name	Jess Edison, MD
Project Role	Co-investigator
Nearest Person Month Worked	3
Contribution	Data extraction; sample management at DoD
Funding Support	Dr. Edison is paid through his position as active duty military and receives no funds from this project.

Name	V. Michael Holers, MD
Project Role	Co-investigator
Nearest Person Month Worked	3
Contribution	Oversee biomarker testing
Funding Support	This project

Name	Ted R. Mikuls, MD/MSPH
Project Role	Co-investigator
Nearest Person Month Worked	3
Contribution	Testing for antibodies to oral pathogens
Funding Support	This project

Name	William Robinson, MD/PhD
Project Role	Co-investigator
Nearest Person Month Worked	3
Contribution	Testing ACPA array

Funding Support	This project
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Name	Jeremy Sokolove, MD
Project Role	Co-investigator
Nearest Person Month Worked	3
Contribution	Testing ACPA array
Funding Support	This project

Name	Geoff Thiele, PhD
Project Role	Co-investigator
Nearest Person Month Worked	3
Contribution	Testing for antibodies to oral pathogens
Funding Support	This project

Name	Gary O. Zerbe, PhD
Project Role	Co-investigator
Nearest Person Month Worked	3
Contribution	Statistical analyses; study design and power
Funding Support	This project

Name	Marie Feser, MSPH
Project Role	Study Coordinator
Nearest Person Month Worked	20
Contribution	Oversee the entire project
Funding Support	This project

Name	Mark Parish, BA
Project Role	Research assistant/laboratory technician
Nearest Person Month Worked	20
Contribution	Laboratory testing/sample management
Funding Support	This project

Name	Emily Stein, PhD
Project Role	Research assistant
Nearest Person Month Worked	3
Contribution	ACPA testing
Funding Support	This project

Name	Lindsay Kelmenson, MD
Project Role	Rheumatology Fellow

Nearest Person Month Worked	4
Contribution	Analyses of final data
Funding Support	University of Colorado Denver Fellowship, NIH T32 and American College of Rheumatology Scientist Development Award.

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period? Please see the following table.

Changes in Active Other Support for PI/Key Personnel During the period of the project	
Kevin Deane, MD/PhD	Dr. Deane has received an appointment at the Denver Veterans Affairs Hospital and received new NIH grant funding; these changes do not affect his effort on this project.
Jess Edison, MD	No changes.
V. Michael Holers, MD	Dr. Holers has received new NIH grant funding and had one grant expire; these changes do not affect his effort on this project.
Ted Mikuls, MD/MSPH	No changes.
William Robinson, MD/PhD	Dr. Robinson has received new NIH funding; these changes do not affect his effort on this project.
Jeremy Sokolove, MD	Dr. Sokolove has received new NIH funding; these changes do not affect his effort on this project.
Geoffrey Thiele, PhD	No changes.
Gary O. Zerbe, PhD	No changes.

What other organizations were involved as partners? There have been no changes from the original proposal in the organizations involved in this project. The organizations that have participated in this project are as follows:

University of Colorado Denver

1775 Aurora Court

Aurora, Colorado USA

The PI Dr. Deane and co-investigator Dr. Holers are based at this institution; all data and samples are housed at this institution.

University of Nebraska Medical Center

986270 Nebraska Medical Center

Omaha, NE 68198 USA

Co-investigators Drs Mikuls and Thiele are based at the University of Nebraska and are performing the testing for antibodies to oral pathogens as well as contributing to the overall design and implementation of the project.

Veterans Affairs Palo Alto Health Care System

3801 Miranda Avenue
Palo Alto, CA 94304

Co-investigators Drs Robinson and Sokolove are based at the Palo Alto VA and are performing testing for the ACPA array and related analyses.

Walter Reed National Military Medical Center
8901 Wisconsin Avenue
Bethesda, MD 20889

Co-investigator Dr. Edison is based at Walter Reed and is obtaining the clinical data and military IRB approvals related to this project.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS

Not applicable.

QUAD CHARTS

Not applicable.

APPENDICES

None